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# Gastrointestinal Health Effects Associated with the Consumption of Drinking Water Produced by Point-of-Use Domestic Reverse-Osmosis Filtration Units

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**During a prospective epidemiological study of gastrointestinal health effects associated with the consumption of drinking water produced by reverse-osmosis domestic units, a correlation was demonstrated between the bacterial counts on R2A medium incubated at 35°C and the reported gastrointestinal symptoms in families who used these units. A univariate correlation was found with bacterial counts on R2A medium at 20°C but was confounded by the bacterial counts at 35°C. Other variables, such as family size and amount of water consumed, were not independently explanatory of the rate of illness. These observations raise concerns for the possibility of increased disease associated with certain point-of-use treatment devices for domestic use when high levels of bacterial growth occur.**

Bacterial growth is a major problem both in water distribution systems (4) and in domestic water filtration units (7, 17). Recently, regulatory agencies in the United States have proposed that the total bacterial counts in finished drinking water should not exceed 500 CFU/ml in order to reduce interference with the detection of coliform bacteria and to reduce subsequent potential health risk (1). Domestic water filtration units are not regulated in terms of the bacterial quality of the water produced, but most manufacturers and regulatory agencies at least attempt to make sure that known pathogens will not be present or allowed to multiply in the units.

During the course of an epidemiological study of the gastrointestinal health effects due to drinking water, reverse-osmosis (RO) water filtration units were installed in randomly chosen households (15). Analysis of the bacterial content of the water filtrated by these units revealed the presence of very high total bacterial counts in most units (14). The present report describes the correlation that was observed between these high bacterial counts and the gastrointestinal illnesses reported during a surveillance period of 10 months for 115 families which used these units and families for which data sets were complete.

## MATERIALS AND METHODS

**Water filtration units.** The under-the-sink RO units were obtained through and installed by a single supplier (Pro-Vie Eau, Inc., Laval, Québec, Canada). They consisted of a sediment prefilter, an activated charcoal filter, an RO membrane (Thin-film composite/Polymer Amarid) rated at 1 to 2 liters/h, a 15-liter reservoir placed under the kitchen sink, and a kitchen sink or countertop faucet. Feed water was obtained by direct connection to the cold-water line, and the RO-membrane wash water (continuous washing) was discharged to the sink drain. The units were installed by professional plumbers, at no expense to the family, in January and February 1988. The sediment and charcoal filters were changed after 9 months of use or sooner if water production decreased, indicating clogging of these filters. The efficiency of the units to remove water contaminants was monitored weekly by a member of the family using an inexpensive conductivity tester (Pro-Vie Eau, Inc.). Water in the reservoir was pressurized by the water pressure of the main inlet to the house and was available at all times from the reservoir in sufficient quantity for normal usage.

**Bacteriological analyses.** The microbiological analyses of water samples from these units have been published elsewhere and have shown that once a device is contaminated, the level of bacterial contamination does not change over time (14). Briefly, data were obtained from 25 units which were under surveillance for 18 months, and from all units, water samples were obtained in September 1988 after units were used for 7 to 9 months. The heterotrophic plate count (HPC) was determined on R2A medium (Difco Laboratories, Detroit, Mich.) by membrane filtration. Colonies were counted after incubation for 7 to 8 days at 20°C or for 48 h at 35°C.

**Health data.** The epidemiological study was carried out in a suburban area of Montreal made up mainly of French Canadians with socioeconomic and education levels similar to the averages for the metropolitan Montreal area (15). In order to determine the level of gastrointestinal illnesses attributable to water, a simple self-administered questionnaire was designed to be completed in 2-week blocks. All families were under surveillance from March 1988 until June 1989 with a break during the summer of 1988. One household member, usually the female head, was designated the informant. The informant was responsible for noting each incident of the following events for any household member on each calendar day: nausea, vomiting, or diarrhea (soft or liquid). If any of these three symptoms was recorded, the presence of the following accompanying symptoms or consequences was to be noted: fever, cramps, muscular pain, cold or flu, sore throat, absence from work or school, visit to a doctor, or hospitalization. At the end of each 2-week period, a nurse telephoned the informant and requested the information recorded in the diary. The data analyzed cover the period of September 1988 to June 1989: the filters had then been in use for more than 6 months. For the purpose of statistical analyses, we defined an episode of highly credible gastrointestinal illness (HCGI) as symptoms involving at

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TABLE 1. Bivariate correlations between HPCs, water consumption, family size, episodes of HCGI, and duration of the episode

| Variable | HCGI[a] | | Days sick[b] | | HPC 20°C[c] | | HPC 35°C[d] | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| HPC 20°C | 0.21 | 0.02 | 0.11 | 0.23 | | | | |
| HPC 35°C | 0.22 | 0.02 | 0.26 | 0.005 | 0.65 | 0.000 | | |
| Water consumption | −0.06 | 0.50 | −0.05 | 0.57 | 0.00 | 0.95 | 0.07 | 0.44 |
| Family size | 0.04 | 0.67 | 0.00 | 0.95 | −0.17 | 0.06 | −0.20 | 0.03 |

[a] Rate of HCGI per person per year.
[b] Number of days sick per person per year.
[c] HPC on R2A medium incubated at 20°C for 8 days.
[d] HPC on R2A medium incubated at 35°C for 2 days.

least one of the following combinations: (i) vomiting or liquid diarrhea with or without confinement to bed, physician visit, or hospitalization or (ii) nausea or soft diarrhea combined with abdominal cramps with or without absence from school or work, confinement to bed, physician visit, or hospitalization. An episode was further defined as consisting of one or more symptomatic days, with at least 6 consecutive symptom-free days between the episodes.

We also carried out a survey to estimate each person's average water consumption habits during a weekday and a weekend day. This questionnaire contained questions about water consumed inside and outside the home and listed different types of use (e.g., straight, in juice, in hot drinks, etc.). From this survey, we computed an estimate of weekly consumption of filtered water and tap water inside and outside the home for each person.

**Statistical analyses.** Estimates of incidence of HCGI were derived by Poisson regression methods (3) and other statistical methods used by our group (15). Because the whole family was exposed to the same level of bacterial contamination, analyses were based on observations of the entire family. A total of 115 families had complete information on both disease and microbiological data for their RO unit and were thus included in the analyses. Regression analyses were performed with both the raw HPCs and their log-transformed values. Since results did not differ, we opted to show only the data on the unmodified counts.

## RESULTS

**Bacteriological content of water samples.** The water produced by most units contained $10^3$ to $10^5$ CFU/ml as previously described (14). The number of bacteria was relatively stable over time as indicated by weekly monitoring of 25 units. The appearance of the bacterial colonies on R2A medium was relatively constant, and most colonies were beige, yellow, or, more rarely, pink. The bacteria isolated were mostly gram negative, rods or cocci, oxidase positive or negative, and mainly of the genera *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Chromobacterium*, *Alcaligenes*, and *Moraxella*. Bacteria isolated at 20°C did not appear to belong to different species than those isolated at 35°C. The bacterial species found in these water samples were similar to those reported by other researchers for activated charcoal filters and domestic water filtration units (7, 17).

**Illness rate and water consumption.** The mean HCGI rate was 0.48 episode per person per year, and the mean number of days sick was 0.89 per person per year. The average consumption at home of unmodified filtered water (not heated or used for cooking) was 5.3 glasses per day per person, or about 750 ml, and the consumption of regular tap water was less than 0.3 glasses per day per person.

**Correlations.** Univariate correlations (Table 1) between the rate of HCGI and the HPCs at 35 and 20°C were both significant at the 5% level. This association suggested that bacteria growing in the filtration units were responsible for an increase in the incidence of gastrointestinal symptoms. There was, however, no apparent correlation between the HCGI rate and the amount of water consumed, suggesting that the statistical correlation with bacterial density might be spurious. A more plausible explanation would be that because of the very high bacterial counts, it was not possible to observe a dose-response relationship, the 100% infective dose already attained for health effects attributable to drinking water consumption. There was no correlation between the length of an episode (number of days sick) and the HPCs at 20°C, but there was a positive correlation with the number of bacteria at 35°C ($r = 0.26$, $P = 0.005$). This meant that the symptoms observed tended to be more severe as the bacterial counts at 35°C increased.

No correlation was found between the amount of filtered water consumed without further treatment (e.g., not heated or used for cooking) and heterotrophic bacteria at 20 or 35°C in filtered water. This implies that there were no negative effects of these bacteria in terms of water quality (e.g., aesthetic quality) causing a possible decrease in water consumption.

TABLE 2. Multiple regression coefficients and associated *P* values (testing the hypothesis that the coefficient is zero) from models for the rate of HCGI symptoms[a]

| Variables removed cumulatively | Regression coefficient (*P* value) | | | | |
|---|---|---|---|---|---|
| | HPC 35°C | Family size | Water consumption | HPC 20°C | Intercept |
| None (complete model) | $3.2 \times 10^{-5}$ (0.056) | 0.13 (0.279) | −0.005 (0.446) | $1.1 \times 10^{-5}$ (0.512) | 0.038 (0.947) |
| HPC 20°C | $3.8 \times 10^{-5}$ (0.003) | 0.13 (0.292) | −0.005 (0.428) | | 0.094 (0.868) |
| Water consumption | $3.8 \times 10^{-5}$ (0.003) | 0.13 (0.281) | | | −0.112 (0.813) |
| Family size | $3.5 \times 10^{-5}$ (0.005) | | | | 0.401 (0.002) |

[a] HPC 35°C, HPC on R2A medium incubated at 35°C for 2 days. HPC 20°C, HPC on R2A medium incubated at 20°C for 8 days.

TABLE 3. Multiple regression coefficients and associated $P$ values (testing the hypothesis that the coefficient is zero) from models for the numbers of days sick

| Variables removed cumulatively | Regression coefficient ($P$ value)[a] | | | | |
|---|---|---|---|---|---|
| | HPC 35°C | HPC 20°C | Water consumption | Family size | Intercept |
| None (complete model) | $2.55 \times 10^{-4}$ (0.006) | $-7.27 \times 10^{-5}$ (0.422) | −0.03 (0.450) | 0.43 (0.523) | 1.516 (0.630) |
| Family size | $2.48 \times 10^{-4}$ (0.006) | $-7.56 \times 10^{-5}$ (0.402) | −0.03 (0.432) | | 3.256 (0.042) |
| Water consumption | $2.42 \times 10^{-4}$ (0.008) | $-7.23 \times 10^{-5}$ (0.422) | | | 2.167 (0.004) |
| HPC 20°C | $1.95 \times 10^{-4}$ (0.004) | | | | 1.948 (0.005) |

[a] HPC 35°C, HPC on R2A medium incubated at 35°C for 2 days. HPC 20°C, HPC on R2A medium incubated at 20°C for 8 days.

A negative correlation was observed between family size and the amount heterotrophic bacteria at 20 and 35°C in filtered water: as more water is drawn from the reservoir, heterotrophs might be diluted by the water produced by the RO membrane. Frequent use of the filtration units thus appears to decrease the number of bacteria in the units.

The HPCs at 20 and 35°C were univariately associated with the HCGI rate and highly correlated with each other. In order to verify whether the two HPC variables exerted independent explanatory effects, we performed multiple regression analyses using both the HCGI rate (Table 2) and the numbers of days sick (Table 3) as dependent variables. This also allowed the analysis of any possible complicating effects of the independent factors under study. Candidate explanatory variables were the two HPCs, family size, and water consumption. Selection of variables was based on a stepwise-backward strategy using a conservative significance level of 0.2.

As shown in Tables 2 and 3, only the HPC at 35°C was a sufficient explanatory variable with respect to both the HCGI rate and the numbers of days sick. In the selection of variables with explanatory value for the HCGI rate, the HPC at 20°C was the first term removed (Table 2). In the models for the numbers of days sick (Table 3), the sign of the regression coefficients of the HPC at 20°C was negative, indicating that the adjusted (by the other factors) association was mostly negative, unlike that seen for the HPC at 35°C. Therefore, the association between disease rate and the HPC at 20°C seen in the univariate analysis seems to be confounded by the much stronger one represented by the HPC at 35°C and disease occurrence, measured by both the rate and the duration of illness.

## DISCUSSION

The symptomatology of gastrointestinal illnesses can be extremely diversified, from highly acute to very discrete according to the etiological agent and the susceptibility of the individual. However, discrete episodes of diarrhea can be caused by toxin-mediated, noninvasive bacterial infections, especially in the elderly, and are often linked to food (16). The species associated with these illnesses are *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and enterotoxigenic *Escherichia coli*. These bacteria are often found in drinking water, but their presence in low numbers is not generally recognized as a health threat.

In fact, many bacteria grow in waters with even minimal nutrient content (9, 17), and they have been found in large numbers in tap-water samples (5, 6, 13) as well as in domestic water filtration units (7, 14). *Legionella pneumophila*, *Pseudomonas aeruginosa*, *B. cereus*, and *Aeromonas hydrophila* are opportunistic pathogens that are regularly found in drinking water, and recent studies have suggested the presence of opportunistic pathogens with virulence characteristics. The nature of the bacterial virulence has been intensively studied for some bacteria, such as salmonellae, escherichiae, and shigellae (2, 8, 10–12, 18), but remains to be studied for most opportunistic pathogens.

Our data suggest that heterotrophic bacteria growing at 35°C in drinking water are associated with low numbers of gastrointestinal illnesses. Numerous species have been found in these water samples, and virulence factors are probably limited to a few of them. The presence of only a few bacterial species with virulence factors might explain the relatively low frequency of symptoms that was observed even in the presence of high bacterial counts. We were probably able to find a correlation because we had a large number of individuals and families under surveillance for almost a year, thus permitting the observation of a large number of episodes with the consequent greater statistical power.

These observations raise concerns about the safety of such point-of-use devices for domestic use and emphasize the importance of controlling bacterial growth in drinking water.

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